One component to this question involves the decreased use of natural products as drugs or drug leads. The movement away from natural products probably reflects the complex synthetic chemistry and medicinal chemistry requirements to optimize delivery and oral bioavailability and to minimize toxicity. Yet natural products tend to produce significant biological activity, presumably because nature has evolved three-dimensional configurations that fit the "grooves and turns" of biochemically important molecules. In addition, many natural products are likely to target multiple species, thereby making the elucidation of their mechanism of action more challenging to dissect.

Although it is difficult to fully define mechanism, we know it when we see it. Mechanism is often presented as a crystal structure of a compound nestled in the crevices of a mutant protein, with hydrogen bonds of the target compound closely approximating critical catalytic residues in the target protein. Polyphenols do not fit this conception of mechanism, because in most cases, including that of fisetin, we don't even know the target protein. In fact, we don't know whether it is a single target protein or multiple target proteins or whether the compounds inhibit protein–protein interactions. We do know that the compound has downstream signaling activities that are important, such as upregulation of E-cadherin and downregulation of c-myc and N-cadherin. Thus, efficacy in the absence of knowledge of preconceived mechanism makes investigators uncomfortable, because it diminishes predictability. Apparently the same may be true of regulatory agencies, such as the Food and Drug Administration in the United States.

Better treatments for melanoma are possible, and they may be right around the corner. Perhaps a rejuvenated interest in natural products would provide a boost—especially if combined with modern methods of target identification, pathway analysis, biomarker discovery, and combinatorial treatments. The power of nature's tools is remarkable. Investigators understood this 40 years ago; revisiting the concept more vigorously might benefit patients.

CONFLICT OF INTEREST
The authors state no conflict of interest.

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Fumarate Esters as Angiogenesis Inhibitors: Key to Action in Psoriasis?

Jack L. Arbiser[1,2,3]

Fumarate esters—an oral therapy for psoriasis—are used primarily in Europe, but not at all in the United States. Given that biological therapies are exceedingly expensive and pose an increased risk for infections and malignancy, the need for safer and less expensive therapies for psoriasis is compelling. Nonbiological therapies for psoriasis, including methotrexate and systemic retinoids, carry potentially severe side effects and relatively high cost. Fumarate, a natural product that is generated internally in humans during the Krebs cycle, is an attractive alternative to these therapies. However, the mechanism for fumarate's activity in psoriasis remains unknown.

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Two reports in this issue of the *Journal of Investigative Dermatology* shed light on possible mechanisms of action of fumarate esters. Meissner *et al.* (2011) demonstrate that dimethylfumarate causes a decrease in tube formation in human endothelial cells *in vitro*. Analysis of angiogenic factors in endothelial cells treated with dimethylfumarate revealed a decrease in vascular endothelial growth factor receptor 2 (VEGFR2) protein but not in VEGFR1 or neuropilin-1. Because VEGFR2 transcription is dependent on the Sp1 transcription factor, the researchers analyzed the effect of dimethylfumarate and demonstrated decreased binding of the Sp1 transcription factor to the VEGFR2 promoter. García-Caballero *et al.* (2011) also demonstrated inhibition of tube formation on Matrigel by dimethylfumarate, but not by monomethylfumarate or free fumaric acid itself. They found that dimethylfumarate does not inhibit the kinase activity of VEGFR2, and they demonstrated antiangiogenic activity in two *in vivo* models: the quail chorioallantoic membrane and a transgenic zebrafish in which the endothelial cells are labeled with green fluorescent protein. Thus, it is safe to say that angiogenesis inhibition probably plays a role in the activity of dimethylfumarate.

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Clinical Implications

- Fumarate esters, which inhibit angiogenesis, are effective in treating psoriasis.
- Fumarate esters, as intermediary compounds in the Krebs cycle, are less like to cause toxicity.
- Fumarate esters are proposed as relatively safe, effective drugs for treating patients with extensive psoriasis.

Several questions remain unanswered concerning the activity of fumarate esters. Although *in vitro* dimethylfumarate is more active than monoesters or fumaric acid itself, it is not known what percentage of dimethylfumarate passes through the digestive tract and serum esterases to reach a psoriatic plaque. Thus, it is uncertain whether dimethylfumarate is the active metabolite *in vivo*. Second, deficiency of fumarate hydratase is associated with leiomyomas and renal cell carcinoma (Martinez-Mir *et al.*, 2003; Pollard *et al.*, 2007; Tomlinson *et al.*, 2002). Fumarate hydratase catalyzes the conversion of fumarate to malate in the Krebs cycle. Deficiencies of several Krebs cycle enzymes are associated with both benign and malignant neoplasms. Other enzymes that have been found to be associated with neoplasia include succinate dehydrogenase and isocitrate dehydrogenase (IDH). In the case of IDH, an oncogenic metabolite, 2-hydroxyglutarate has been proposed to account for the increase in tumors related to IDH deficiency, but in the case of fumarate hydratase and succinate dehydrogenase, the associated oncogenic metabolites are not known (Dang *et al.*, 2010). Critical to our understanding of carcinogenic mechanisms associated with Krebs cycle deficiencies is whether the defect is cell autonomous or nonautonomous and whether carcinogenesis is attributable to intracellular metabolites of fumarate or high concentrations of extracellular fumarate. The lack of cancers, especially renal cell carcinoma, in patients taking dimethylfumarate for long periods suggests that high levels of extracellular fumarate do not mediate the carcinogenesis seen in fumarate hydratase deficiency.

Let us assume that fumarate is the active metabolite of dimethylfumarate. What is known about its mechanisms of action? Part of the proposed means of cancer pathogenesis, in the face of Krebs cycle deficiencies, is an upregulation of hypoxia-inducible factors (HIF1a and HIF2a) in the presence of normoxia (called "pseudohypoxia") (Dang *et al.*, 2010). The consequence of upregulating HIF is transcriptional induction of vascular endothelial growth factor (VEGF). Notably, although HIF1a and HIF2a are highly homologous, activation has different consequences; in a study of renal cell carcinoma, the presence of HIF2a was found to be associated with an aggressive phenotype, whereas HIF1a overexpression is associated with a less aggressive phenotype, suggesting potential tumor suppressor activity of HIF1a (Gordan *et al.*, 2008). In a murine model of fumarate hydratase deficiency, both HIF1a and HIF2a are induced as part of neoplasia. However, the addition of endogenous fumarate induces HIF1a but not HIF2a (MacKenzie *et al.*, 2007). Thus, there is a difference between the effects of fumarate hydratase deficiency and large quantities of exogenous fumarate (Yogev *et al.*, 2010). The most likely model to explain fumarate's beneficial activity is through induction of HIF1a (Figure 1), leading to downregulation of NF-κB and Sp1; precedence for an anti-inflammatory effect of HIF1a has been demonstrated through induction of heme oxygenase-1 (Belcher *et al.*, 2006; Chin *et al.*, 2007).

Fumarate has been useful in treating psoriasis, and there is reason to believe it may be effective in treating other inflammatory disorders. It is fortunate that fumarate has already been approved for human use, because in today's regulatory climate it would likely not be approved, especially as a nontargeted therapy. Further studies of fumarate esters' mechanism of action are warranted because they could lead to safe and effective systemic therapies for psoriasis and other inflammatory disorders.

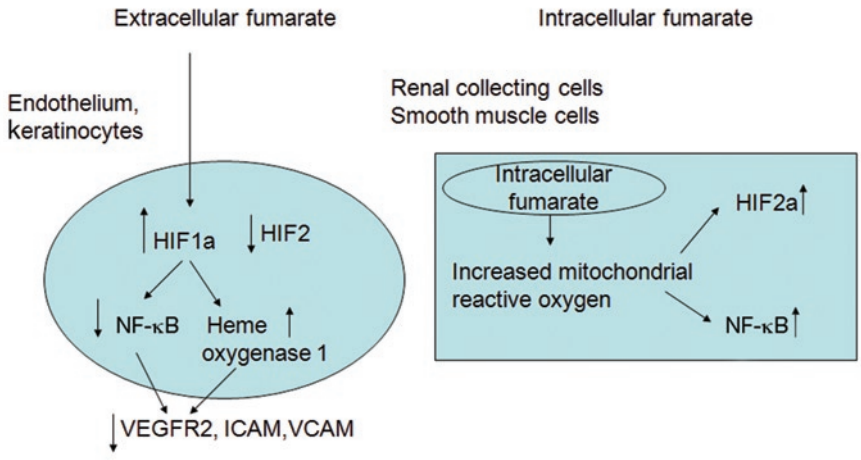

Figure 1. Proposed mechanisms of action for extracellular and intracellular fumarate. Extracellular fumarate may cause an induction of hypoxia inducible factor (HIF) 1a but downregulation of HIF2a. Anti-inflammatory effects of HIF1a induction may be mediated through downregulation of NF-κB and upregulation of heme oxygenase 1. In renal collecting duct cells, fumarate hydratase deficiency may lead to upregulation of both HIF1a and HIF2a through a reactive oxygen–dependent pathway. ICAM, intercellular cell adhesion molecule; VCAM, vascular cell adhesion molecule; VEGFR2, vascular endothelial growth factor receptor.

CONFLICT OF INTEREST

The author states no conflict of interest.

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